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17-DMAG, an Hsp90 inhibitor, ameliorates ovariectomy-induced obesity in rats

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ABSTRACT

Aims: Obesity is not only associated with metabolic diseases but is also a symptom of menopause in women. To date, there are no effective drugs for the management of obesity, and it is important to find new agents with fewer side effects, for the treatment of obesity. This study aimed to determine the anti-obesity effect of 17-dimethylaminoethylamino-17-demethoxygeldanamycin (17-DMAG), a heat shock protein 90 (Hsp90) inhibitor, and its underlying mechanism in rats with ovariectomy-induced obesity.

Main methods: Ovariectomy (Ovx) rats were treated with 17-DMAG (1 mg kg^{-1}, intraperitoneally) for eight weeks from one week after surgery. The body weight, food intake, locomotor activity, adipogenic- and autophagy-related protein expression in white adipose tissue (WAT) and plasma triglyceride (TG) levels were measured in sham and Ovx rats.

Key findings: Compared with sham rats, Ovx rats showed increased weight gain, food intake, WAT mass, TG levels, adipogenic protein expression, and decreased locomotor activity. Furthermore, autophagy-related proteins and Foxo3a of WAT were significantly increased in Ovx rats. However, with the exclusion of increased food intake, the changes induced by Ovx were all reversed in 17-DMAG-treated Ovx rats. In addition, the expression of Hsp70 and phosphorylation of Akt increased in 17-DMAG-treated Ovx rats.

Significance: These results suggest that 17-DMAG significantly ameliorated obesity induced by Ovx, and this phenomenon is accompanied by the downregulation of adipogenic-related and autophagy-related proteins as well as the upregulation of Akt-phosphorylation and Hsp70 expression. Therefore, 17-DMAG may be a potential agent for preventing or treating obesity in postmenopausal women.

1. Introduction

Obesity has become an important problem in developing countries, and its prevalence has increased significantly worldwide [1]. Menopause, the age-related loss of ovarian hormone production, is associated with increased visceral adiposity and related metabolic pathologies including insulin resistance, type 2 diabetes, and cardiovascular disease [2–5]. The personal and public health effects of menopause-associated disease are significant, because women can now expect to spend nearly a third of their lives in the menopausal state. To date, no effective and safe treatment has successfully reduced the prevalence of menopause-associated obesity; therefore, the development of innovative treatments and prophylaxis remains of great interest. Obesity is characterized by the accumulation of white adipose tissue (WAT). The traditional role of

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WAT is energy storage, and fatty acids are released from the WAT when fuel is required [6]. When WAT stores excess energy, hyperplasia (cell number increase) and hypertrophy (cell size increase) occurs. During adipogenesis, new adipocytes form from precursors, and these pre-adipocytes differentiate into mature adipocytes [7]. Several transcription factors play important roles in adipogenesis, such as peroxisome proliferator-activated receptor γ (PPARγ), CCAAT/enhancer-binding protein α (C/EBPα), fatty acid binding protein (FABP4), fatty acid synthase (FAS), and lipoprotein lipase (LPL).

Autophagy is an important cytoplasmic degradation pathway through which macromolecules and organelles are degraded by lysosomes [8]. Recent research revealed that autophagy is activated when primary mouse fibroblasts mature into adipocytes [9]. The pre-adipocytes go through many subcellular morphological changes during adipocyte differentiation, such as increased cytoplasmic volume, emergence of endoplasmic reticulum, and formation of lipid droplets [10]. Additionally, many mitochondria are encircled in autophagosomes during adipogenesis, which shows that autophagy plays a role in cytoplasm remodeling [11]. To create space for the storage of lipid droplets, cytosolic organelles and protein complexes are sequestered in a double-membrane autophagosome vesicle, which fuses with lysosomes and is broken down by lysosomal enzymes [12,13]. Autophagy is highly controlled by a serine/threonine-protein kinase known as mammalian target of rapamycin (mTOR), and mTOR activity is regulated by several upstream kinases [14]. Akt activates mTOR and inhibits autophagy through activation of the upstream mTOR regulator, tuberous sclerosis complex 1/2 (TSC1/2) [15]. In addition, several studies show that heat shock protein 70 (Hsp70) may also play a regulatory role in autophagy [15,16]. Hsp70-induced Akt phosphorylation leads to mTOR phosphorylation, which blocks autophagy [17].

The Hsp90 inhibitor, 17-DMAG, is an analog of geldanamycin, and several studies have shown the anti-inflammatory and anti-cancer effects of 17-DMAG [18–21]. The activity of Hsp90 is reduced when17-DMAG binds to the Hsp90 ATP-binding site. Hsp70 expression is induced by Hsp90 inhibitors through the activation of heat shock factor-1 (HSF-1) [21–24]. The aim of this study was to investigate whether 17-DMAG could ameliorate obesity induced by ovariectomy (Ovx) via the activation of Hsp70 and subsequent decrease of autophagy in rats.

2. Materials and methods

2.1. Animal preparation

Female Wistar rats (8 weeks old) were obtained from BioLASCO Taiwan Co., Ltd. The rats were weighted and subjected to either sham or bilateral Ovx operations after anesthetizing with Zoletil® (20 mg kg^{-1}, intraperitoneally [i.p.]; Virbac Co, Carros, France) [25]. After surgery, the rats were housed with water and food freely available and maintained in 12 h light/dark cycles and temperatures between 20 and 26 °C. The research was conducted in accordance with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (Publication No. 85-23, revised 1996) and approved by the National Defense Medical Center Institutional Animal Care and Use Committee, Taiwan. At the end of the experiments, to verify the Ovx, the serum estrogen levels were checked using chemiluminescence immunoassay (ASC:180, Bayer Diagnostics, Tarrytown, NY, USA) on an automated analyzer. The estrogen levels averaged 72.7 ± 6.4 pg mL^{-1} and 51.5 ± 1.3 pg mL^{-1} in the sham and Ovx groups, respectively ($p < 0.05$; $n = 3$ for each group).

2.2. Experimental groups

The rats were grouped into sham, Ovx, and Ovx + 17-DMAG ($n = 8$–11). Rats in the sham and Ovx groups received normal saline (1 mL kg^{-1} day^{-1}, i.p.) while rats from the Ovx + 17-DMAG group were treated with 17-DMAG (1 mg kg^{-1} day^{-1}, i.p.) (InvivoGen, San Diego, CA, USA) beginning 1 week after the operation. All the groups were treated for 8 weeks, with food intake and body weight measured weekly. At the end of the experiment, rats were killed by administering 50 mg kg^{-1} Zoletil i.p. Mesenteric and perirenal adipose tissue samples were immediately excised, weighed, frozen in liquid nitrogen, and stored at −80 °C for later analysis.

2.3. Serum determination

Whole blood was collected and centrifuged for 6 min at 12,000 × *g*. Serum triglycerides were determined by Fuji DRI-CHEM 303 analyzer (Fuji Photo Film Co., Tokyo, Japan).

2.4. Locomotor activity

At the end of the experiments, the rats were placed in a separate cage and their total distance traveled in 60 min recorded. The data were analyzed with TopView Behavior Analyzing System [26].

2.5. Western blot analysis

The adipose tissue was homogenized in RIPA buffer containing phenylmethylsulfonyl fluoride and phosphatase inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), then centrifuged at 8000 ×*g* for 20 min at 4 °C. Protein concentration was quantified with the BCA protein assay kit (Thermo Fisher Scientific, MA, USA). Proteins were separated by electrophoresis on SDS-polyacrylamide gels and transferred to nitrocellulose membranes. The blots were incubated with primary antibodies at 4 °C overnight. The primary antibodies used comprised of rabbit anti-PPARγ, anti-LPL, anti-FAS, anti-FABP4, anti-Beclin-1, anti-p62, anti-C/EBPα, anti-LC3B, anti-phospho-Akt, anti-Akt, anti-Foxo3a (all from Cell Signaling Technology, Danvers, MA, USA), mouse anti-Hsp90, anti-Hsp70 (Enzo Biochem Inc., NY, USA), and anti-β-actin (Sigma-Aldrich). After washing, the membranes were probed with the corresponding second antibodies. The signal was detected as described in previous study [25].

2.6. Statistical analysis

The data are presented as the means ± standard errors of the mean (SEM). Statistical evaluation was performed by a one-way analysis of variance followed by the Holm–Sidak multiple comparisons test. Differences with $p < 0.05$ were considered significant.

3. Results

3.1. Effect of 17-DMAG on body weight in Ovx rats

The mean body weights of the rats in the three groups are shown in Fig. 1A. At the time of operation, all rats had similar body weights (162 ± 1.5 g). The body weight of Ovx rats progressively increased and reached a maximum (337.75 ± 4.9 g) at 9 weeks after Ovx (Fig. 1A). The body weight of these rats was significantly higher than that of rats in the sham group (Ovx, 337.75 ± 4.9 g vs. sham, 273.12 ± 5.2 g, $p < 0.05$). However, the body weight of rats in the Ovx + 17-DMAG group was significantly lower than that of rats in the Ovx group (Ovx + 17-DMAG, 305 ± 6.7 g vs. Ovx, 337.75 ± 4.9 g; $p < 0.05$).

3.2. Effect of 17-DMAG on food intake in Ovx rats

The food intake for all rats was measured once a week. As shown in Fig. 1B, the food intake of rats within each group did not differ at the time of operation or one week after Ovx. However, food intake was significantly higher than that of the sham group two weeks after Ovx in both the Ovx and Ovx + 17-DMAG groups (Ovx, 163.6 ± 5.6 g $week^{-1}$ per

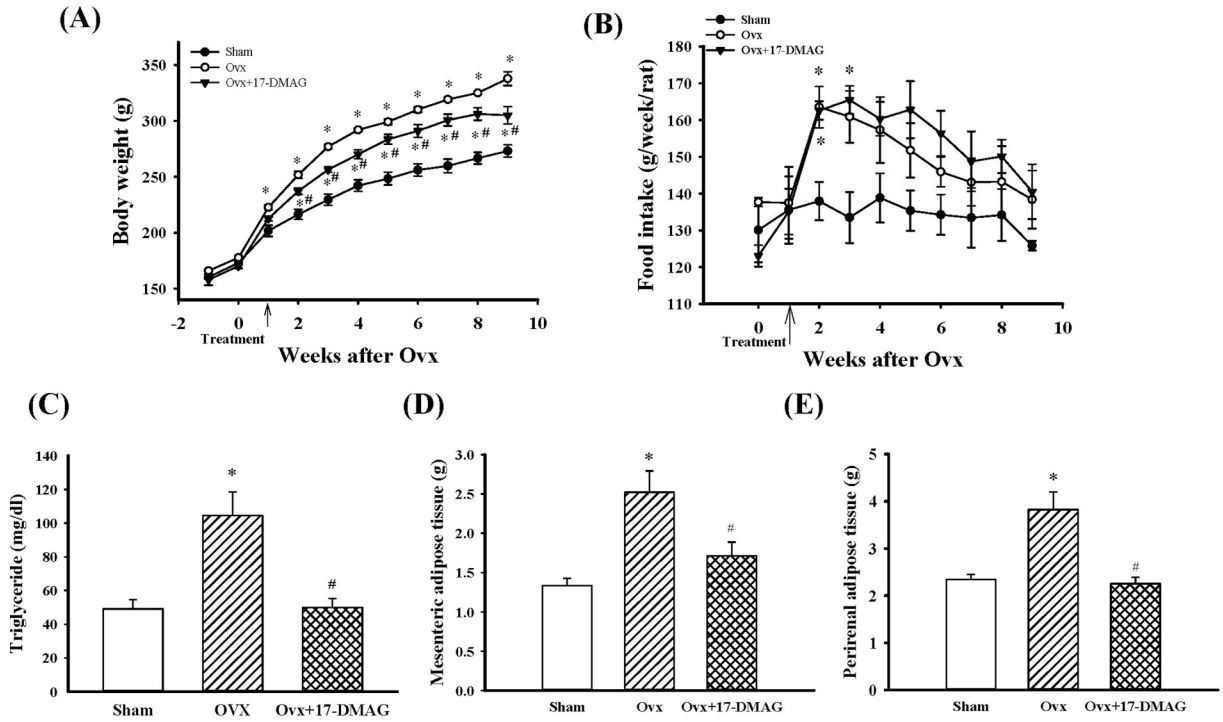

Fig. 1. Effect of 17-DMAG in rats with ovariectomy (Ovx)-induced obesity. (A) Body weight, (B) food intake, (C) triglyceride levels in the serum, and (D) weight of mesenteric and (E) perirenal adipose tissues of rats. Data are expressed as the means ± standard error of the mean (n = 8–11). *$p < 0.05$ compared to the sham group; #$p < 0.05$ compared to the Ovx group.

rat; Ovx + 17-DMAG, 162.6 ± 2.5 g week^{-1} per rat, vs. sham, 138 ± 5.2 g week per rat) and then progressively decreased to a similar level as the sham group.

3.3. Effect of 17-DMAG on serum triglyceride in Ovx rats

As shown in Fig. 1C, the triglyceride level of rats in the Ovx group was significantly higher than that of rats in the sham group (Ovx, 104.5 ± 14 mg dL^{-1} vs. sham, 49 ± 5.6 mg dL^{-1}; $p < 0.05$). However, the triglyceride level of rats in the Ovx + 17-DMAG group was significantly lower than that of rats in the Ovx group (Ovx + 17-DMAG, 49.8 ± 5.5 mg dL^{-1} vs. Ovx, 104.5 ± 14 mg dL^{-1}; $p < 0.05$).

3.4. Effect of 17-DMAG on WAT weight in Ovx rats

As shown in Fig. 1D and E, both the mesenteric and perirenal adipose tissue weight were significantly higher in Ovx rats than in sham rats ($p < 0.05$). However, the adipose tissue weight was significantly lower for 17-DMAG-treated Ovx rats than for Ovx rats ($p < 0.05$).

3.5. Effect of 17-DMAG on locomotor activity in Ovx rats

Locomotor activity is a simple and easily performed measurement of behavior in rodents and was used to investigate whether energy expenditure was influenced by 17-DMAG treatment in Ovx rats. In the locomotion test, the distance traveled per 5 mins was not significantly different among groups (Fig. 2A), and the total distance traveled in 60 min by Ovx rats was significantly shorter than that traveled by sham rats (Fig. 2B; $p < 0.05$). In addition, the total distance traveled by Ovx + 17-DMAG rats was significantly more than that by Ovx rats ($p < 0.05$) but not significantly different from the distance traveled by sham rats (Fig. 2B).

3.6. Effect of 17-DMAG on expression of adipogenesis proteins in the perirenal adipose tissue of Ovx rats

To explore the mechanisms underlying the anti-lipogenic effect of 17-DMAG, the perirenal adipose tissue was used to analyze lipogenic protein expression. As shown in Fig. 3A–D, the expression of PPARγ, FAS, LPL, and FABP4 protein levels in the perirenal adipose tissue of the Ovx rats were significantly higher than that of the sham rats ($p < 0.05$). However, these protein expression levels were significantly lower in the Ovx + 17-DMAG group than in the Ovx group ($p < 0.05$).

3.7. Effect of 17-DMAG on the expression of autophagy-related proteins in the perirenal adipose tissue of Ovx rats

Autophagy is an important factor involved in adipocyte differentiation; therefore, the expression of autophagy-related protein levels of WAT was analyzed. As shown in Fig. 3E and F, the expression of Beclin-1 protein was significantly higher and that of p62 protein was significantly lower in the perirenal adipose tissue of Ovx rats than that in the perirenal adipose tissue of sham rats ($p < 0.05$). However, the pattern of expression of these proteins was significantly reversed in the Ovx + 17-DMAG group compared to the Ovx group ($p < 0.05$).

3.8. Effect of 17-DMAG on expression of adipogenesis proteins in the mesenteric adipose tissue of Ovx rats

As shown in Fig. 4A–C, the lipogenic proteins PPARγ, C/EBPα, and FABP4 of mesenteric adipose tissue were significantly higher in Ovx rats than in sham rats ($p < 0.05$). These protein levels were significantly lower in the Ovx + 17-DMAG group than in the Ovx group ($p < 0.05$).

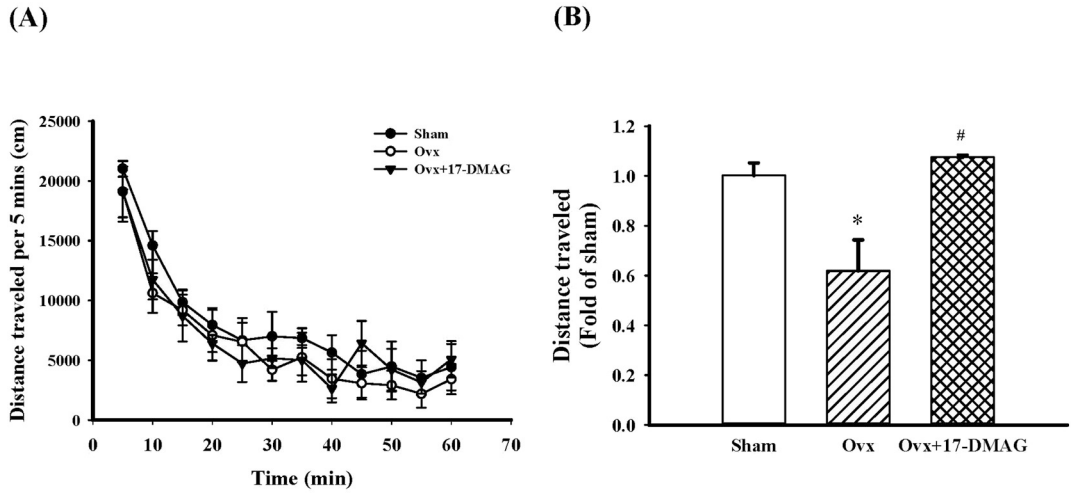

Fig. 2. Effect of 17-DMAG on locomotor activity in ovariectomy (Ovx) rats. (A) Locomotor activity of rats during a 60 min period, recorded with 5 min intervals, and (B) cumulative locomotor activity in Ovx rats during the 60 min period. Data are expressed as the means ± standard error of the mean ($n = 5$). *$p < 0.05$ compared to the sham group; #$p < 0.05$ compared to the Ovx group.

3.9. Effect of 17-DMAG on expression of autophagy-related protein in the mesenteric adipose tissue of Ovx rats

As shown in Fig. 4D and E, the LC3BII/LC3BI ratio and p62 degradation were significantly higher in the mesenteric adipose tissue of Ovx rats than sham rats ($p < 0.05$). The pattern of expression of these proteins was reversed in the Ovx + 17-DMAG group compared with the Ovx group ($p < 0.05$).

3.10. Effect of 17-DMAG on the expression of heat shock protein in the perirenal adipose tissue of Ovx rats

As shown in Fig. 5A, Hsp90 protein expression of perirenal adipose tissue was significantly higher in both the Ovx and Ovx + 17-DMAG

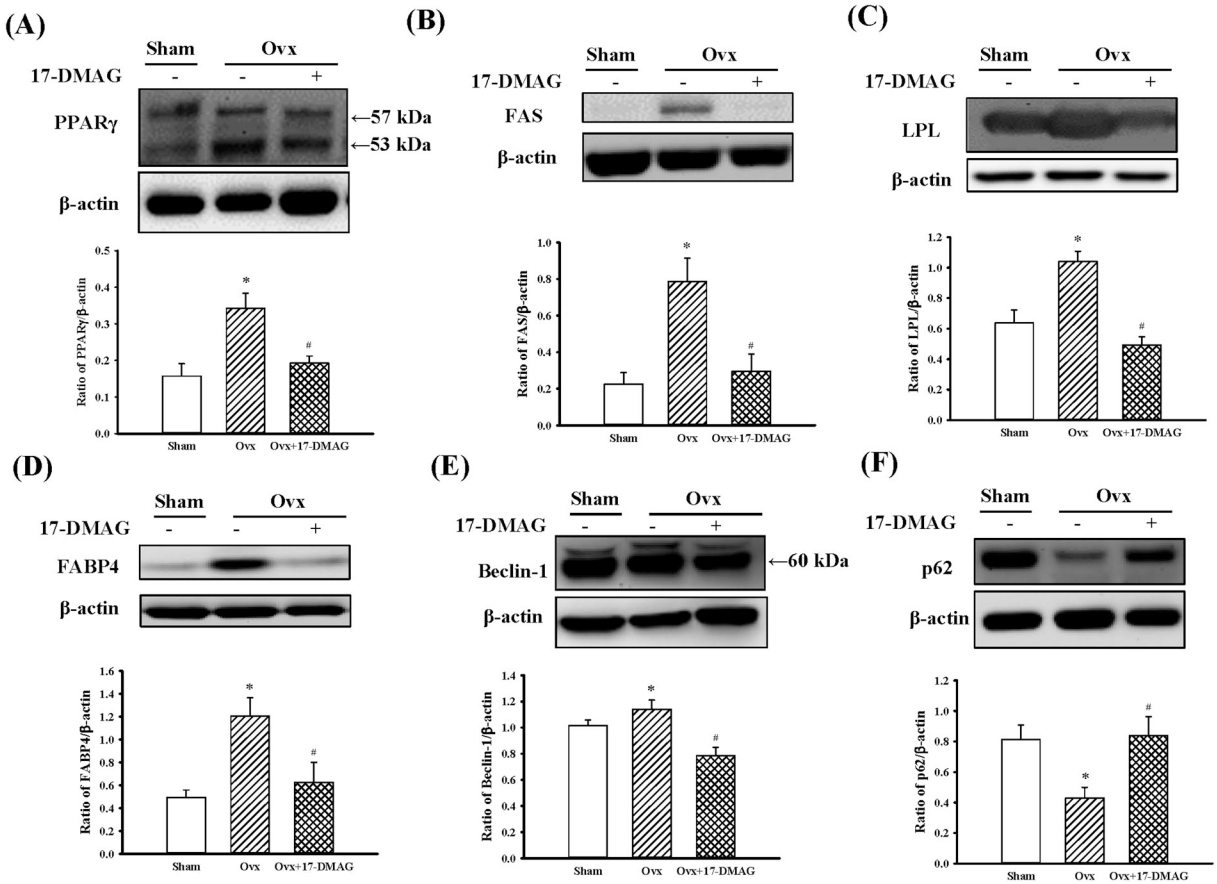

Fig. 3. Effect of 17-DMAG on the expression of adipogenic proteins and autophagy-related proteins in perirenal adipose tissue in ovariectomy (Ovx) rats. Expression of adipogenic proteins (A) PPARγ, (B) FAS, (C) LPL (D) FABP4, and autophagy-related proteins (E) Beclin-1 and (F) p62 were analyzed by western blotting. Data are expressed as the means ± standard error of the mean ($n = 3$–5). *$p < 0.05$ compared to the sham group; #$p < 0.05$ compared to the Ovx group.

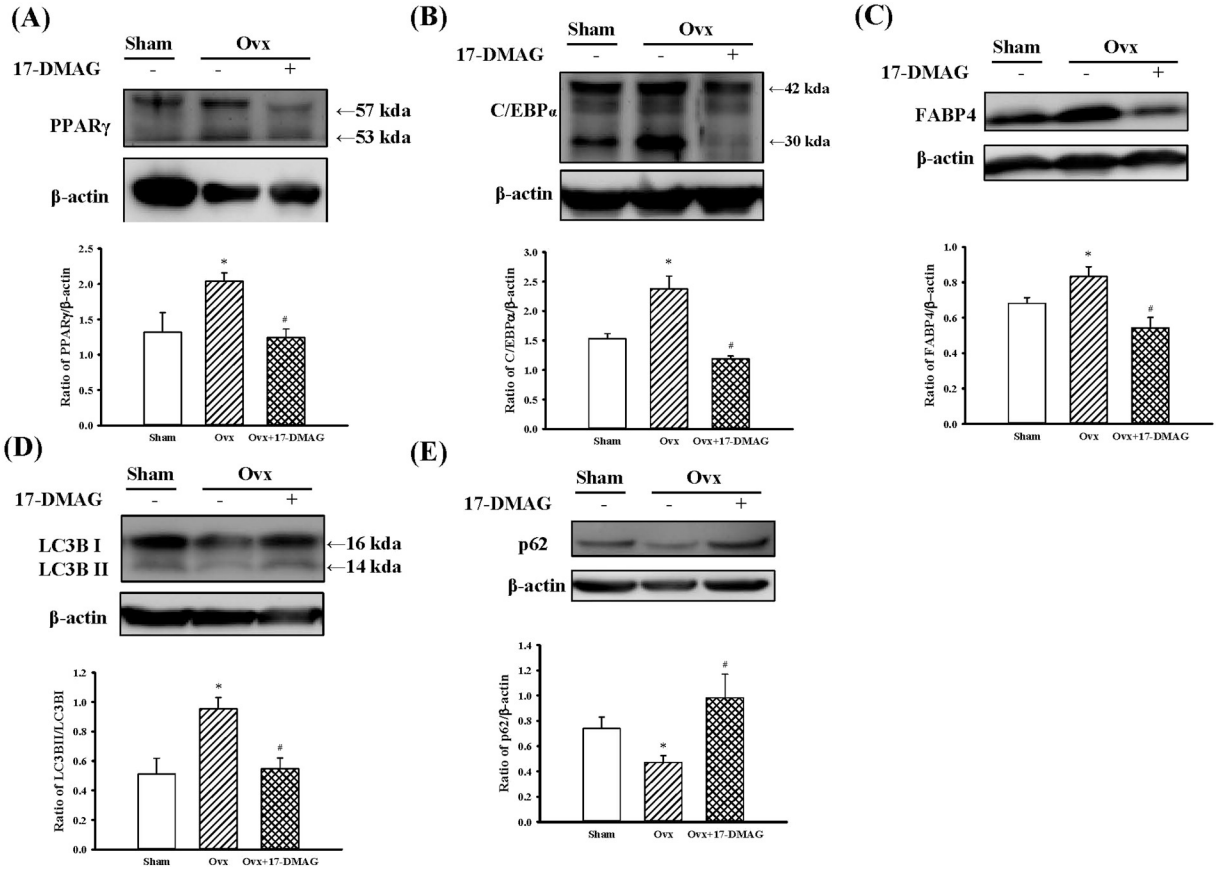

Fig. 4. Effect of 17-DMAG on the expression of adipogenic proteins and autophagy-related proteins in mesenteric adipose tissue in ovariectomy (Ovx) rats. The adipogenic proteins (A) PPARγ, (B) C/EBPα, and (C) FABP4, and autophagy-related proteins (D) LC3B, and (E) p62 were analyzed by western blotting. Data are expressed as the means ± standard error of the mean (n = 3–5). $^{*}p < 0.05$ compared to the sham group; $^{\#}p < 0.05$ compared to the Ovx group.

groups than in the sham group ($p < 0.05$). In addition, the expression of Hsp70 in the perirenal adipose tissue of Ovx + 17-DMAG group was significantly higher than that in the sham and Ovx groups ($p < 0.05$).

3.11. Effect of 17-DMAG on the expression of p-Akt and Foxo3a in the perirenal adipose tissue of Ovx rats

As shown in Fig. 5C, the phosphorylation of Akt was significantly higher in the perirenal adipose tissue of rats in the Ovx + 17-DMAG group than in the sham and Ovx groups ($p < 0.05$). In addition, the expression level of Foxo3a in the perirenal adipose tissue was significantly higher in Ovx group than in the sham group (Fig. 5D; $p < 0.05$). The expression of Foxo3a was significantly lower in the Ovx + 17-DMAG group than in the Ovx group (Fig. 5D; $p < 0.05$).

3.12. Effect of 17-DMAG on the expression of heat shock proteins in the mesenteric adipose tissue of Ovx rats

As shown in Fig. 6A, the expression of the Hsp90 protein was significantly higher in the mesenteric adipose tissue of the Ovx and Ovx + 17-DMAG groups than in the sham group ($p < 0.05$). The expression of Hsp70 was significantly higher in the Ovx + 17-DMAG group than in the sham and Ovx groups ($p < 0.05$).

3.13. Effect of 17-DMAG on the expression of p-Akt and Foxo3a in the mesenteric adipose tissue of Ovx rats

As shown in Fig. 6C, the phosphorylation of Akt was significantly higher in the mesenteric adipose tissue of rats in the Ovx + 17-DMAG group than in the sham and Ovx groups. In addition, the Foxo3 expression was significantly higher in the Ovx group than in both sham and Ovx + 17-DMAG groups (Fig. 6D).

4. Discussion

Ovariectomy in rodents is used as a model of obesity after menopause [27,28]. Several studies indicate that estradiol-deficiency causes weight gain, increased food intake, and accumulation of body fat [29,30]. In addition, high blood triglyceride levels and body fat accumulation are a major risk factor for cardiovascular diseases [31]. The animal model has also been confirmed in our previous experiments conducted by our group [32,33]. In the present study, the weight gain of Ovx rats significantly decreased after 17-DMAG treatment for 8 weeks (Fig. 1A). Additionally, 17-DMAG reduced triglyceride levels and the weight of mesenteric and perirenal adipose tissue in Ovx rats even though food intake was not affected. Thus, the anti-obesity effect of 17-DMAG may not result from the inhibition of appetite.

Maintaining energy balance depends on the efficiency of tightly regulated mechanisms of energy intake and expenditure. Obesity is ultimately the result of a chronic positive imbalance between these two factors. Locomotor activity is usually used for measuring movement distance in rats to quantify activity [34]. Several studies have shown that Ovx rats have lower levels of energy expenditure and locomotor activity [35–37]. Human data also indicate a reduction in 24 h energy expenditure during the menopausal transition and decreased physical activity [38]. In the present study, the total distance traveled by rats in the Ovx + 17-DMAG group was more than that of rats in the Ovx group (Fig. 2B). Therefore, the change in body weight is not parallel with the food intake but may be associated with the physical activity in Ovx + 17-DMAG rats. In addition, we found that the adiposity (fat pad

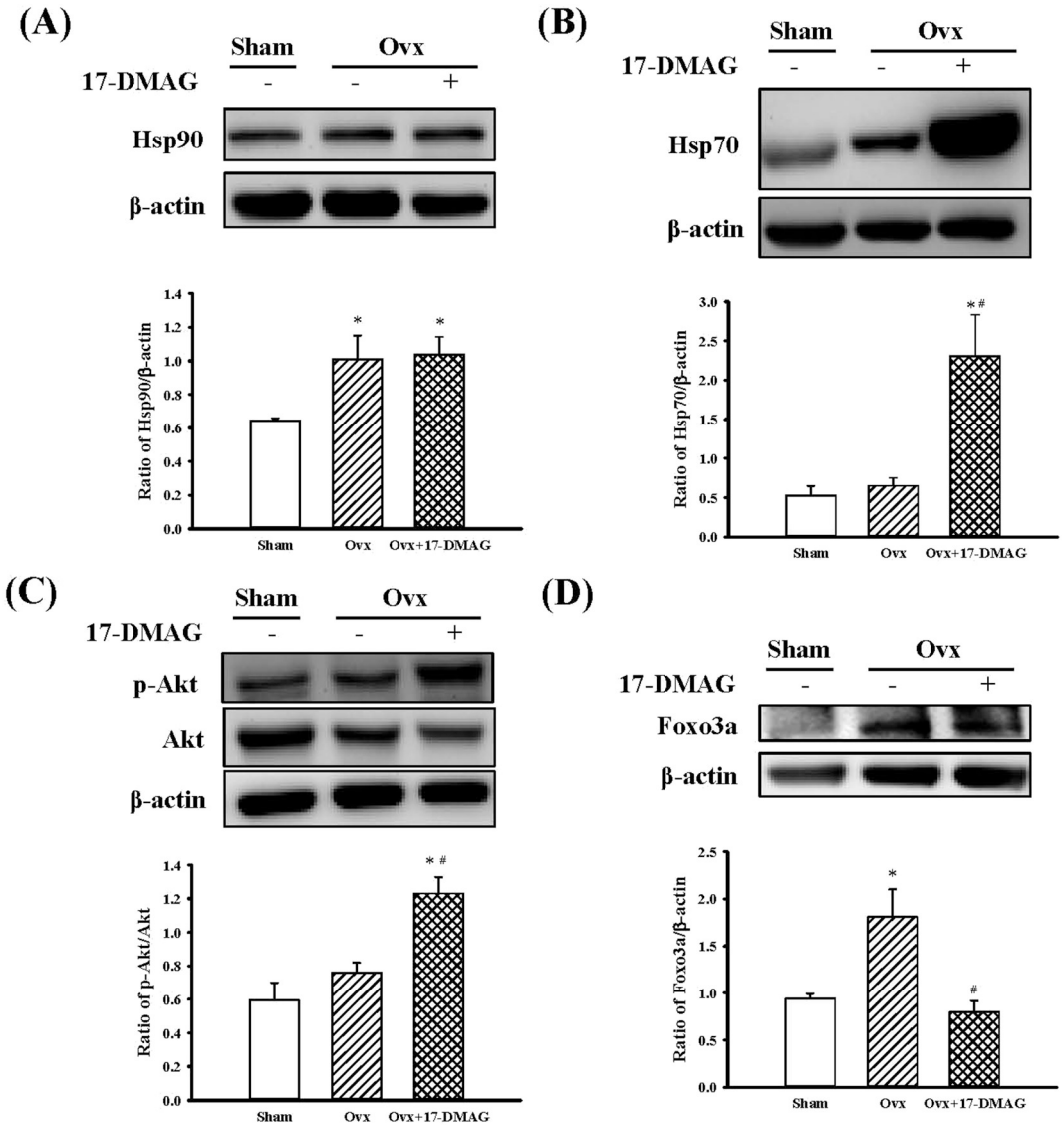

Fig. 5. Effect of 17-DMAG on the expression of (A) Hsp90, (B) Hsp70, (C) phosphorylated Akt, and (D) Foxo3a proteins in the perirenal adipose tissue of ovariectomy (Ovx) rats. Data are expressed as the means ± standard error of the mean (n = 3–5). $^{*}p < 0.05$ compared to the sham group; $^{\#}p < 0.05$ compared to the Ovx group.

weight) was completely normalized by 17-DMAG; however, the body weight was only partially normalized. This may have resulted from the direct and/or indirect effects of 17-DMAG on the adipogenesis, physical activity, and metabolic rate. This speculation is supported by previous studies showing that Hsp90 inhibitor prevented adipocyte differentiation and reduced lipid accumulation [39–41]. Further studies are required to clarify the relationship of 17-DMAG with physical activity and metabolic rate.

PPARγ and C/EBPα are two known transcription factors that regulate the process of adipogenesis [42]. In the present study, 17-DMAG significantly decreased the expression of PPARγ and C/EBPα (Figs. 3A, 4A and B.), after that the downstream target proteins LPL, FAS, and FABP4 (Figs. 3B–D and 4C) decreased in the visceral adipose tissue. These results suggest that downregulation of adipogenic proteins by 17-DMAG may result in attenuation of fat accumulation in Ovx rats. In addition, several studies have shown that autophagy plays an important role in cytoplasmic remodeling during adipogenesis. Autophagy deficient primary atg5-/- mouse embryonic fibroblasts exhibited significantly reduced efficiency in adipogenesis [9]. The targeted deletion of Atg7 results in less gonadal WAT than that in wild type mice [43,44]. In the present study, the expression patterns of the autophagy-related proteins induced by Ovx in the perirenal and mesenteric adipose tissue were all reversed by 17-DMAG treatment (Figs. 3E and 4D). In addition, the level of p62, a protein for cargo selection and protein aggregation, is usually inversely correlated with autophagic degradation. Therefore, p62 can be used as an autophagy marker [45]. In the present study, p62 protein levels of mesenteric and perirenal adipose tissue increased after 17-DMAG treatment in Ovx rats (Figs. 3F and 4E). These results indicate that 17-DMAG decreased fat accumulation, which may be associated with the downregulation of adipogenesis and autophagy-related proteins. Further studies are required to clarify the relationship of 17-DMAG with autophagy flux.

Apart from autophagy-related proteins, there are many regulators involved in autophagy. Activated Akt positively regulates the activity of mTORC1 with consequent autophagy inhibition [15]. In addition, Akt activation inhibits Foxo3 activation and autophagy independently of mTOR [46]. In the present study, the Akt phosphorylation significantly increased, whereas Foxo3a expression significantly reduced in perirenal and mesenteric adipose tissue of Ovx rats after treatment with 17-DMAG (Fig. 5C–D, and Fig. 6C–D). These findings imply that 17-DMAG regulates the autophagy necessary for adipogenesis by modulating Akt and Foxo3a. Interestingly, Hsp70 plays a regulatory role in autophagy.

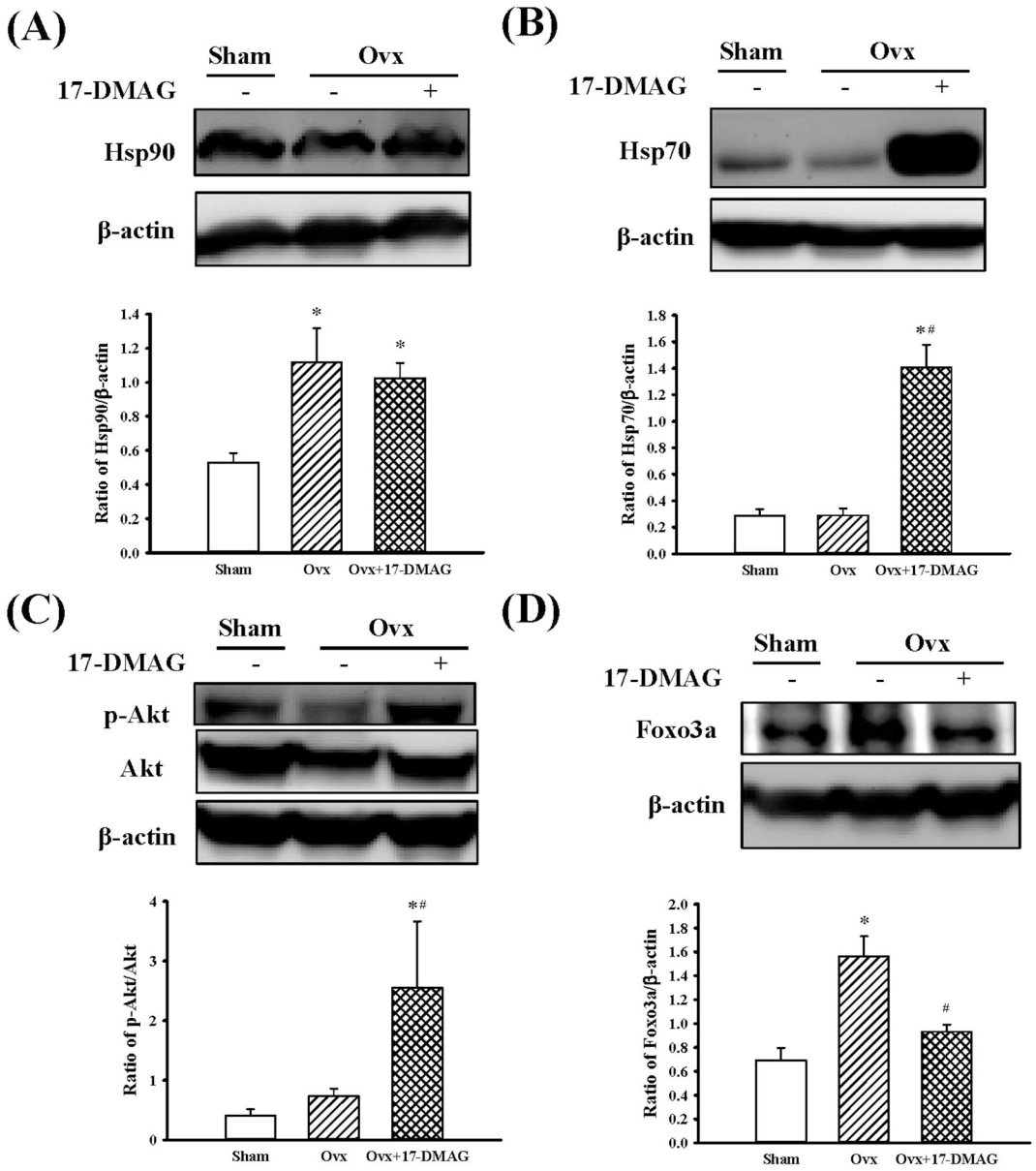

Fig. 6. Effect of 17-DMAG on the expression of (A) Hsp90, (B) Hsp70, (C) phosphorylated Akt, and (D) Foxo3a proteins in mesenteric adipose tissue of ovariectomy (Ovx) rats. Data are expressed as the means ± standard error of the mean (n = 3–5). $^{*}p < 0.05$ compared to the sham group; $^{\#}p < 0.05$ compared to the Ovx group.

Hsp70 inhibits autophagy induced by the mTOR inhibitor rapamycin, suggesting that Hsp70 regulates autophagy through the Akt and mTOR pathway [16]. In addition, it has been reported that Hsp90 inhibitors are associated with the activation of HSF-1 and lead to the activation of Hsp70 [18,21,47]. In the present study, Hsp70 protein expression was significantly increased in the mesenteric and perirenal adipose tissue of 17-DMAG-treated Ovx rats. This supports that 17-DMAG attenuates the expression of autophagy-related proteins through the induction of Hsp70 and Akt phosphorylation. Although we have no direct evidence to explore the mechanism, it does not rule out the possibility that Hsp70 plays an important role in the regulation of autophagy in Ovx rats (Fig. 7).

It is noteworthy that the expression of Hsp90 was significantly increased in WAT of Ovx rats in this study. This may be explained by the consistent increase in Hsp90 with increases in estrogen receptor after Ovx [48]. It has been proposed that receptor-associated proteins maintain their receptors in a conformation that results in a high affinity for a hormone [49]. In addition, 17-DMAG is considered to be an Hsp90 inhibitor via binding to the N-terminal domain of Hsp90 and disassociating Hsp90-HSF1 complex, resulting in activation of HSF1 [50]. Hsp70, one of the transcriptional targets of HSF1, has been reported to regulate autophagy by Akt and mTOR pathway. Therefore, it is expected to observe both increased Hsp90 and Hsp70 expression in WAT of 17DMAG-treated Ovx rats, but not in saline-treated Ovx rats. However, further studies are needed to clarify whether the anti-obesity effect observed with 17-DMAG is due to Hsp70 up-regulation, inhibition of Hsp90 activity, or both.

In conclusion, our results showed that 17-DMAG treatment exerts an anti-adipogenic effect associated with activation of Hsp70, phosphorylation of downstream Akt, suppression of Foxo3a, downregulation of autophagy-related protein expression, and inhibition of fat accumulation in rats with Ovx-induced obesity. In addition, 17-DMAG attenuated body weight gain, adipose tissue weight, and triglyceride levels in rats with Ovx-induced obesity. These effects were associated with increased energy expenditure by increasing locomotor activity as well as the expression of proteins associated with adipogenesis and autophagy. Thus, 17-DMAG might be a potential therapeutic drug for the prevention and treatment of obesity in postmenopausal women. These findings were

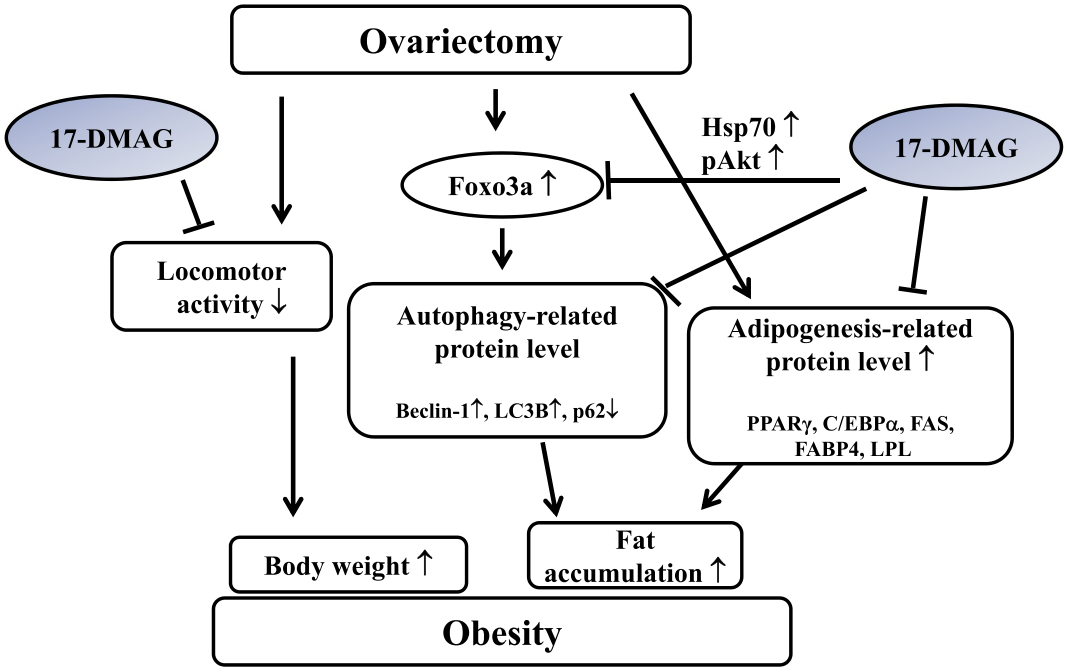

Fig. 7. Suggested effects associated with 17-DMAG in rats with ovariectomy-induced obesity. C/EBPα, CCAAT/enhancer-binding protein α; FABP4, fatty acid binding protein; FAS, fatty acid synthase; LPL, lipoprotein lipase; PPARγ, peroxisome proliferator-activated receptor γ. (→) Stimulatory and (⊥) inhibitory action.

strengthened by those of a previous study showing that 17-DMAG prevented body weight gain and fat mass expansion in mice fed a high-fat diet [39], thereby indicating a potential clinical application for 17-DMAG in obesity. Regarding possible future in vivo application, the different effects on other forms of obesity cannot be excluded and the relevance of the tested dosage is unknown, even though the dose used in this study is much lower than the maximum tolerated dose [51]. Thus, many more details remain to be evaluated before 17-DMAG can be recommended in the treatment of obesity.

Declaration of Competing Interest

The authors declare that they have no competing financial interests.

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